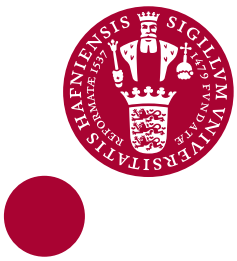

How does a plant orchestrate defense in time and space? Using glucosinolates in Arabidopsis as case study

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How does a plant orchestrate defense in time and space? Using glucosinolates in Arabidopsis as case study

Meike Burow and Barbara Ann Halkier

The sessile nature of plants has caused plants to develop means to defend themselves against attacking organisms. Multiple strategies range from physical barriers to chemical warfare including pre-formed anticipins as well as phytoalexins produced only upon attack. While phytoalexins require rapid induction, constitutive defenses can impose ecological costs if they deter pollinators or attract specialized herbivores. In the model *Arabidopsis thaliana*, the well-characterized glucosinolate anticipins are categorized into different classes, aliphatic and indole glucosinolates, depending on their amino acid precursor. Using glucosinolates in Arabidopsis as case study, we will discuss how plants orchestrate synthesis, storage and activation of pre-formed defense compounds spatially and temporally.

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Introduction

Specialized plant metabolites are well-known for their role in interactions between plants and their distinct biotic environments. Consequently, and as opposed to general metabolites, specialized metabolites are restricted to specific taxa. Their enormous diversity in chemical structures, biosynthetic pathways, and modes of action [1,2] has evolved under selection pressure imposed by specific combinations of harmful and beneficial interacting organisms. Chemical defense strategies in plants range from constitutive accumulation over induced defenses to activated defense systems. The latter depend on at least two components—the preformed, inactive precursor of a bioactive compounds and the activating enzyme machinery. For the plant to ensure that appropriate chemical defenses are present in the right tissue and at the right time, their biosynthesis, transport and storage needs to be tightly regulated in space and time. As different defense strategies underlie different defense pathways, a general discussion of their spatio-temporal regulation is troublesome. Thus, this review focuses on glucosinolates as model for activated chemical defenses.

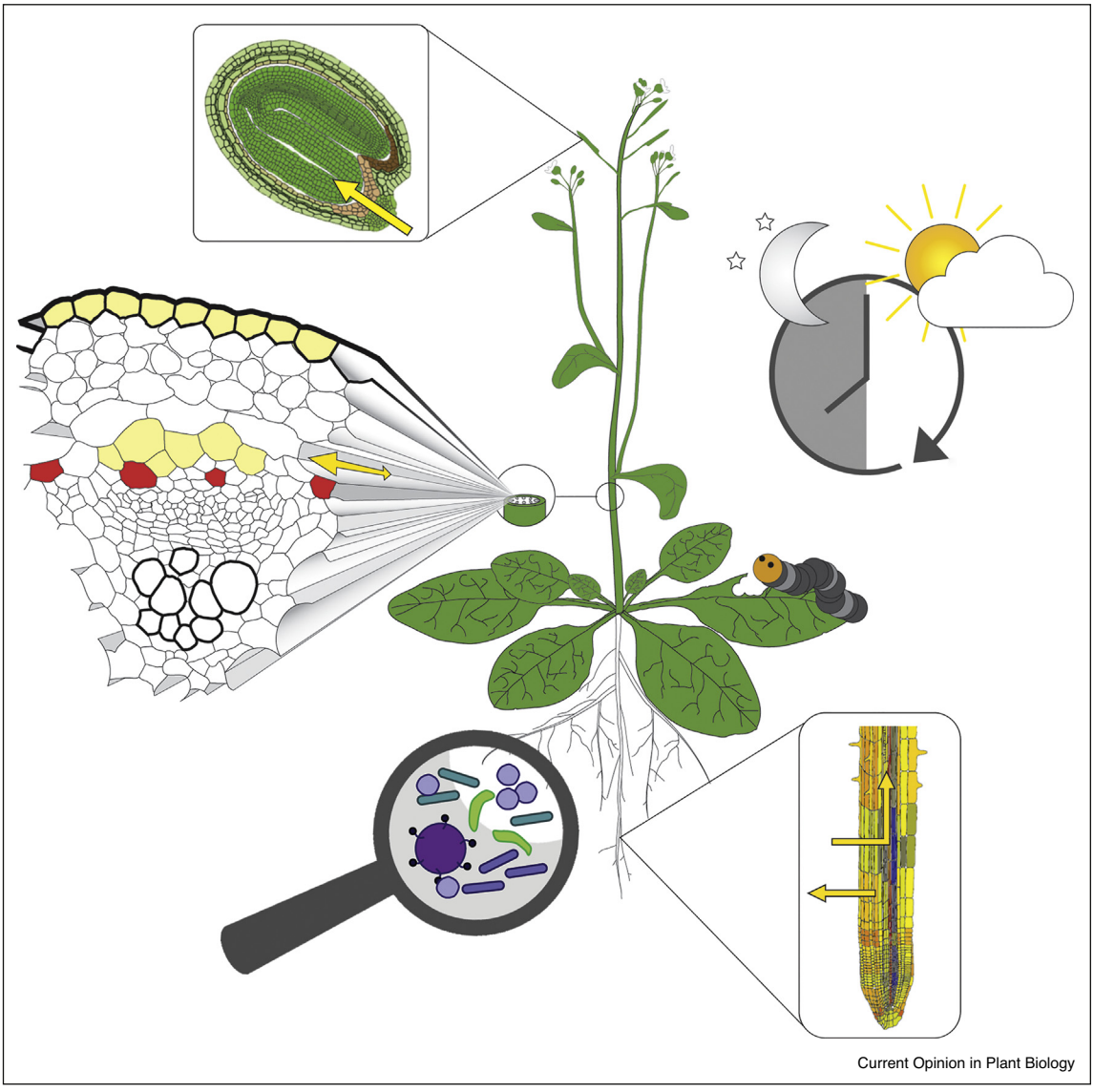
As primary chemical defense compounds, Arabidopsis synthesizes up to 40 different glucosinolates [3]. The glucosinolates themselves show only very limited biological activities, and depend on activation initiated by co-occurring thioglycosidases called myrosinases. Chemical and enzymatical rearrangements after myrosinase-catalyzed hydrolysis give rise to a range of products toxic to a wide range of organisms [1,4]. Depending on the amino acid-derived glucosinolate side chain and the presence of specifier proteins, each glucosinolate can be activated to one or more products with different biological activities [5]. Variation in the type of glucosinolate hydrolysis products is observed among different species within the Brassicaceae, among Arabidopsis accessions and even among tissues of the same plant [6–8]. Upon herbivory, glucosinolate levels can be further induced and induction of the nitrile-specifier protein NSP1 can change the outcome of glucosinolate hydrolysis and thereby shift a direct to an indirect defense strategy [9,10]. This underlines the versatile and dynamic nature of glucosinolate-based defense.

Storage of glucosinolates in S-cells and seeds

In the unattacked Arabidopsis plant, glucosinolates are stored in laticifer-like S-cells within the phloem cap region outside the vasculature and along the leaf margin [11–15] (Figure 1). These cells contain >130 mM glucosinolates stored under high turgor pressure. Although laticifer-like cells are known from, for example, rubber trees it remains an open question whether the localization of laticifer-like cells (such as the S-cells) in the phloem cap region is a Brassicales-specific evolution or whether universally found for other defense compounds.

Other storage sites include the seeds that accumulate high levels of glucosinolates that are imported into the seeds that lack *de novo* biosynthesis [16,17]. Knowledge about the seed loading process is limited. The so-far only identified glucosinolate transporters, the plasma

Figure 1



Spatio-temporal control of glucosinolate-based defense. Like numerous other specialized metabolites, glucosinolates play a key role in plant biotic interactions, both above- and below-ground. Their presence and activation in the right tissue at the right time is critical for their biological functions. Glucosinolate profiles characteristic for a given tissue, developmental stage and combination of environmental factors are dynamically shaped by biosynthesis and transport. Along vascular bundles, rapid activation upon tissue damage relies on close proximity of glucosinolate storage cells (S-cells shown in yellow in the schematic stem cross section) and myrosin cells (shown in red).

membrane-localized glucosinolate transporter 1 (GTR1) and glucosinolate transporter 2 (GTR2) importers, are essential for loading glucosinolates into the seeds as demonstrated by glucosinolate-free seeds of the *gtr1 gtr2* mutant [18]. The level of glucosinolates in a given tissue is subject to a complex feedback regulation. Plants overexpressing the MYB28 – a major positive regulator of aliphatic glucosinolates – accumulated threefold higher levels of aliphatic glucosinolates in the foliar tissue, while their seeds showed only a moderate increase [19]. Thus, independent of the levels of glucosinolates in vegetative parts, the levels in the seeds appear to reach a certain maximum. It is currently not known how the plant senses that the seeds have reached this threshold.

Sinks within the leaves

Glucosinolate concentration in leaves decreases with age until virtually gone upon senescence [8]. Whether the glucosinolates are (re)mobilized to sink tissue or turned over is unknown. It was suggested that leaf glucosinolates were destined to the seeds based on increased levels in leaves of *gtr1 gtr2* mutant [18]. However, it turned out that

the overaccumulation in leaves was due to glucosinolates derived from roots via the xylem [20,21]. This suggests that upon senescence the leaf-synthesized glucosinolates are not transported long distance to the seeds, but rather turned over. How leaf-synthesized glucosinolates are turned over in the intact leaves upon senescence is a puzzle, although glucosinolate breakdown independent of the classical myrosinases thioglucoside glucohydrolase 1 (TGG1) and thioglucoside glucohydrolase 2 (TGG2) has been shown [22]. By contrast, the root-derived glucosinolates accumulating in leaves in the *gtr1 gtr2* mutant [21] are apparently turned over at much lower rate. Possibly, glucosinolates that arrive via xylem accumulate in different sites than the leaf-synthesized ones.

Recently, the cuticular leaf layer was identified as glucosinolate sink [23•]. The surface localization of glucosinolates has been a controversial topic for many years as various methods have yielded ambiguous results. Recently, Shroff *et al.* [23•] used three independent mass spectrometry methods to elegantly detect and quantify intact glucosinolates at the surface. In addition to previous findings, where glucosinolates were shown to accumulate along the major midrib and the leaf margin [24], the surface-localized glucosinolates represent 1–5% of total glucosinolates and are evenly distributed across the epidermis, except for the midrib [23•]. The distribution between adaxial and abaxial surfaces was found to be uneven with 15–30-fold more on the abaxial surface. How glucosinolates get exported out of epidermis to the cuticula is not known. Noticeably, particularly 4-methylthiobutyl glucosinolate (4MTB) (eightfold) and indole-3-methyl glucosinolate (I3M) (threefold) are overrepresented in both epidermis [21] and cuticula [23•]. The significance of this is not known, but the levels are sufficient to function as oviposition cues [23•].

The rhizosphere is a sink

Recently, intact glucosinolates [25] as well as hydrolysis products [25,26] were detected in the exudate of Arabidopsis roots, as they have previously been detected in *Brassica* root exudates [27,28]. Intuitively, one would have anticipated that rhizosecreted phytochemicals were produced in the outer cell layers of the root. However, in Arabidopsis the route from site of synthesis to the rhizosphere required GTR-mediated import of stele-synthesized glucosinolates into the symplasm [25]. This indicated that glucosinolates must be exported from the cell in which they are synthesized and that GTR1 and GTR2 might be essential in balancing above- and below-ground defense in response to environmental challenge. The effects of *Brassica* root exudates in suppression of soil-born pests have primed an interest in use of glucosinolate-containing plants in pest management, a process termed biofumigation [29]. The endophyte *Piriformospora indica* requires a certain level of glucosinolate hydrolysis to maintain its status as commensalistic/mutualistic endophyte [30], which indicates that the impact of intact glucosinolates and their hydrolysis products on the microbial community in the rhizosphere represents an interesting future research area.

Are glucosinolates mobilized upon attack?

Until now we have discussed glucosinolates as phytoanticipines that accumulate in various sinks in the unchallenged plant. However, upon attack by biotrophic pathogens, for example, *Blumeria* and *Phytophthora* species, cell-autonomous defense linked to indole glucosinolates has been shown to play role a plant innate immunity [31,32]. Upon such attacks, unmodified indole glucosinolates I3M produced along the vasculature [33] and stored in the epidermal cell [21] are modified by the induced enzyme CYP81F2 to produce 4-methoxy-indole glucosinolate [34]. It is currently not known whether the epidermal pool of I3M is being replenished by *de novo* synthesis in the epidermal cell or whether the vasculatory synthesis sites deliver upon attack? Also, where in the unchallenged epidermal cell is the pre-formed I3M stored? Is it in the vacuole where glucosinolates are normally stored? If so, how does it get remobilized to the cytosol to encounter the endoplasmic reticulum-associated CYP81F2? Future studies are required to address these questions related to the orchestration of synthesis and storage as well as remobilization and replenishment when under attack.

How do plants coordinate development and defense?

During development, fluxes through metabolic pathways constantly need to be adjusted to account for changes in source-sink relations [35,36]. In the winter-annual Arabidopsis, the emergence of the florescence coincides with the onset of senescence in rosette leaves which marks an essential development transition. Decreasing levels of glucosinolates in senescing leaves [8] have been attributed to the emergence of the inflorescence as a new source tissue [37], but may as well be a consequence of changes in general metabolism including reduced availability of precursors and increased remobilization of nitrogen. At the same time, higher levels in young and reproductive tissues and lower levels in senescent tissues represent a typical developmental pattern for chemical defenses reflecting re-allocation of resources within the plant and its impact on the behavior of herbivores as well as that of pollinators and other beneficial organisms [38].

Thus, the networks controlling development and defense must be intimately linked to balance metabolic versus ecological costs and thereby maximize plant fitness. And indeed, the same phytohormones provide input to development, growth and regulate chemical defenses [39] supporting a view in which plant specialized metabolism is just another metabolic output of the complex signal transduction networks driving highly conserved biological processes. Despite the metabolic costs associated with

specialized metabolites, the relationship between growth and defense is not necessarily a tradeoff [40]. Instead, molecular decisions made to coordinate growth and defense are determined by the abiotic environment including the availability of nutrients, in the case of glucosinolates most importantly nitrogen and sulfur [41••].

What mediates feedback regulatory effects of specialized metabolites?

As levels of chemical defenses are strongly intertwined with developmental processes and seasonal changes in the biotic and abiotic environment, they can be expected to provide feedback regulatory input to these developmental processes to maximize the plant's ability to efficiently utilize the resources available. In line with such a model, genetic variation in the glucosinolate biosynthetic *alkenyl- and hydroxyalkyl-producing* locus (*GS-AOP*) was associated with variation in the onset of flowering [42,43] and one of the genes in the *GS-AOP* locus, *AOP2*, is involved in regulatory loop linking jasmonate signaling and glucosinolate biosynthesis [44•]. The identification of *gulliver1/superroot2-7* – a weak allele of the cytochrome P450 CYP83B1 involved in the biosynthesis of indolic glucosinolates from tryptophan – further highlighted the established link between indole glucosinolate and indole acetic acid synthesis and hinted at a checkpoint for the coordination of the two pathways [45]. Even specific glucosinolate structures can have feedback regulatory effects as illustrated by naturally variable biomass responses in Arabidopsis seedlings specifically to the methionine-derived allyl glucosinolate, a product of the enzymatic activity of AOP2 [46•,47]. The molecular mechanisms underlying structure-specific fine-tuning of growth and development by glucosinolates and potentially other specialized metabolites remain, however, to be uncovered.

What are the mechanisms linking circadian clocks and defense?

A multitude of developmental and physiological processes are controlled through cross-talk with molecular clocks as central regulators. Like any other multicellular organism, plants have circadian clocks to coordinate their metabolism with environmental factors by integrating biotic and abiotic factors [48,49]. In plants, this coordination is critical for local adaptation because it allows plants to anticipate predictable fluctuations in the environment and adjust development and physiology accordingly through phytohormone signaling pathways [50–53]. Also pollinators, herbivores, pathogens rely on endogenous clocks and therefore represent – at least to some extent – predictable biotic threats, as indicated by the timing of plant immune responses and increased resistance to herbivores and pathogens that coincides with the highest insect and pathogen activity [54,55].

Depending on temperature, basal levels of root glucosinolates have been reported to show a circadian pattern [56], however, quantitative changes in glucosinolate levels are relatively low and it remains to be tested how much these oscillations contribute to day time-dependent differences in plant resistance. On the level of transcriptional control, the circadian clock and glucosinolates show clear interconnectivity, again at least partially mediated by known regulators of glucosinolate biosynthesis from methionine [43]. Arabidopsis mutants with altered levels of *AOP2* or the transcriptional regulators *MYB28* and *MYB29* show a significantly altered circadian period providing yet another example of an output of the clock functioning as feedback regulatory input.

Future research should aim at identifying the parameters that determine whether or not oscillations in transcript levels translate into changes in metabolite levels. Whereas circadian control of glucosinolate transcripts reflects the plant's ability to anticipate herbivore and pathogen attack, diurnal changes in signaling pathways and general metabolism might be decisive factors for basal and inducible levels of defense compounds and thereby plant resistance. The central role of clock components in integrating biotic and abiotic external signal with information from internal feedback loops leaves no doubt about its impact on the dynamics of chemical defenses, but neither about future challenges in identifying the molecular mechanisms behind.

Biotechnological use of circadian clock to promote postharvest longevity

The modular design of plants enables individual plant organs to manifest autonomous functions and continue aspects of metabolism, for example, respiration, even after separation from the parent plant. Accordingly, harvested vegetables and fruits continue to sense and respond to diverse stimuli, similarly to intact plants. In a recent study, circadian clock entrainment with light/dark cycles during postharvest storage improved plant tissue performance with respect to tissue integrity, green coloration, and chlorophyll content, compared to constant light or darkness [57•]. In the cruciferous vegetables, kale and cabbage, the levels of the glucosinolates remained at higher levels when stored under light/dark cycles. This suggests that sustained circadian clock entrainment may be a powerful approach to promote postharvest quality and longevity and thereby reduce yield loss. By applying this finding to postharvest storage, more food could be kept for longer without refrigeration.

Conclusion

The complexity of the orchestration of chemical defenses in plants reflects the complexity of the environments that have shaped the underlying regulatory networks. To understand how plants balance metabolic and ecological

costs to support both development and defense under fluctuating conditions requires detailed knowledge on the spatial and temporal dynamics of synthesis, storage, and mobilization of chemical defenses. We can expect the same knowledge to inspire future biotechnological strategies for improved food quality.

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- •• of outstanding interest

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